Invitro study on the antibacterial effect of Plant Derived nanoparticles (PDNPs) synthesised from ethnobotanical plants found in Palghar district against some pathogenic bacterial strains.

A Research Proposal

Submitted to

University of Mumbai

For the degree of

DOCTOR OF PHILOSOPHY

(Botany)

By

Mrs. Lisa Sam

INDEX

Sr. No.	CONTENT	Pg. No.
1.	Introduction	2
2.	Review of Literature	6
3.	Rationale	17
4.	Aims and Objectives	20
5.	Plan of work for proposed study	21
6.	Instruments and Apparatus to be used in the study	23
7.	Expected outcomes	24
8.	Contribution to society	24
9.	Future prospects	25
10.	References	26

INTRODUCTION

India is a botanical garden of the biosphere and a gold mine of well-recorded and traditionally well practised knowledge of the herbal remedy. **(Singh SP. et. al, 2003)** There are significant economic benefits in the expansion of indigenous medicines and the practice of medicinal plants for the management of several diseases. India has a rich flora with an extensive diversity of plants, because of the extreme dissimilarities in physical and climatic conditions prevailing in the nation. A large segment of the Indian population still depends on traditional medicinal plants as they are abundantly existing, inexpensive and effective.

Ethnobotany- in its wider context- denotes the entire realm of the beneficial relationship between plants and men **(Arora 1997)**. Ethnobotany deals with the complex relationships between (uses of) flora and culture(s), focusing on how plants have been or are used, managed, and perceived in human societies, including plants used for dietary consumption, medicine, divination, cosmetics, dyeing, textiles, building, tools, currency, clothing, rituals, and social life (**Rahman 2009**). The ancient use of many alternative medicine practices, including experience passed down from generation to generation, has proven the safety and efficacy of conventional medicine. Plants are either used as indigenous therapy or as isolated active principles for a common source of medicine. Indigenous ethnopharmacology has been considered as an important tool in the exploration of new medicines. Herbal medicines are a common method of complementary and alternative medicine used on the treatment of various forms of ailments around the globe. Medical herbalism was advocated due to its rarer side effects and reported efficacy against many diseases. Traditionally all medicinal preparations were derived from plants, whether in the simple form of plant parts or the more sophisticated way of crude extracts, mixtures, etc. Crude herbal formulations claimed to have major medicinal benefits with or without additives. In the discovery of new lead molecules, synergistic herbal formulations used by the traditional healers can be used as essential tools. There is currently a universal renaissance of ethnobotanical surveys of medicinal plants and the necessity for screening precise parts of plants.

(**Ashidi J.S. et. al, 2010)**

As per the research article, '**Documentation And Ethnobotanical Survey Of Wild Edible Plants From Palghar District', by Mahadkar Shivprasad* et. al, 2016** the study in the Palghar district revealed that about 40 varieties of plant species of which leaves, flowers, inflorescence, tubers, bulbils and fruits are mainly used for consumption.

Palghar district is aptly known as the home of aboriginals who are locally known as 'Adiwasis'. They account for 25.40 percent of the total district population and are mostly concentrated in rural areas. This proportion is the second largest among the districts in Maharashtra. The district scheduled tribe population accounts for 19.62 per cent of the corresponding total in the State. Scheduled tribe population is found in considerable numbers throughout the district though mostly concentrated in Dahanu, Jawhar, Shahapur, Talasari and Mokhada talukas.

The tribal communities in Palghar also depend on the WEP (Wild Edible Plants) for their dietary and medicinal purposes apart from the agricultural produce. These WEP are largely harvested from the local forest areas present around villages. One of the major issues faced by the tribal population in this region is malnutrition. Malnourishment remains a major problem in the region mostly in the women and children below the age group of 6 years. **Studies by Hatekar and Rode., 2003; Dimri et.al, 2012** have reported the issue of malnourishment to be severe in case of infants and children. One of the possible reasons for this severity could be lack of knowledge about healthy diet, ignorance towards wellbeing and proper sanitation or lack of food variety and absence of purchase power to procure nutritious food. Due to this the tribal population especially the children are more prone to diseases. The unhygienic conditions and improper sanitization has led to worsen the conditions.

Inorder to bring about a noteworthy improvement in the life of the tribal communities, proper scientific research has to be taken up to in these areas wherein the local natural treasure can be explored and tapped for it beneficial aspects for the local community in general without exploiting the natural resources indiscriminately.

Nanotechnology involves the synthesis of materials that are in nanometre sizes (1 to 100 nm). Nanotechnology has appeared as a new area of fundamental science and is getting worldwide consideration due to its extensive applications. They have exceptional physicochemical properties due to their unique size, shape and size distribution. Conventionally nanoparticles were synthesised using physical and chemical methods. These methods have various disadvantages therefore biological methods possess great interest. Biological synthesis uses bacteria, fungi, algae and plants. Biosynthesis of nanoparticles using plants emerges as nanofactories as they offer nontoxic, clean and eco-friendly methods with various physicochemical properties**. (Roy et. al, 2020)** The occurrence and prevalence of various diseases have been rising on an ever-increasing scale in the past few years. This is due to a number of factors leading to the reduced susceptibility of the infection causing agents to the existing drugs in the market. Micro-organisms are the sources of numerous diseases that exist in the environment. These microbes have been studied and relevant antibiotics and vaccines have been developed to contain the spread of the diseases among the human population. Antibiotic medications are used to kill bacteria, which can cause illness and disease. They have made a major contribution to human health. Many diseases that once killed people can now be treated effectively with antibiotics. However, increasing variations in bacterial response to many existing drugs have been observed over the last few years. Antibiotics being indiscriminately prescribed by the health professionals is also a major cause of the problem. Besides, the inappropriate use of the prescribed drugs and lack of awareness about the upcoming threats due to such activities among the local population is another major factor. The fear of side-effects occurring due to various drugs also lead to the incomplete treatments for a particular disease condition.

The nanoparticles synthesised to date have two major limitations:

i) each constituent of the synthesised nanoparticles must be examined for potential *in vivo* toxicity before clinical application; and

ii) the production scale is limited.

In contrast, nanoparticles derived from natural sources are considered to be safe and cost effective that may overcome aforementioned limitations of synthetic nanoparticles. **(S. Babu, L. M. et. al, 2011).**

REVIEW OF LITERATURE

Plants are providing human kind with all needs including food, clothing, shelter, flavours and fragrances as not the least, medicines. Plants form the basis of traditional medicine, Ayurvedic, Unani, Chinese amongst others. Medicinal plants have been used throughout the world, however, their wide usage has been limited to China, India, Japan, Pakistan, Sri Lanka, Thailand and African countries. Developed countries are also turning to encourage the usage of plant-based natural medicinal products in their healthcare systems. The World Health Organisation estimates that 80% of the world's inhabitants rely mainly on traditional medicines for their health care **(Farnsworth, 1994; Mukherjee and Wahil, 2006).**

Based on the observations by **Reddy, 2012; Bhogaonkar et. al, 2010**, it is understood that the wild plants make an important contribution to the life of rural communities. Wild edible plants are those with one or more parts that can be used for food if gathered at the appropriate stage of growth and properly prepared. Tribal people fulfil their nutritional requirements from wild resources. The tribes have shown dependence, mainly on the wild plant species for purposes such as food, medicine, shelter, and so on.

Further, **Chothe et. al, 2014; Nene, 2004** have observed that the wild plants have not only proven to be the source of food for daily consumption, but also as a key resource, during extreme situations, such as famines as well.

Apart from the dietary aspect, the village doctors, locally called as Vaidu, who are aware of the medical properties of the wild edibles, have been successful in providing remedies for several ailments, at the village level, studied **D'souza, 1999 and Palekar, 1993**.

Thus, the wild plants have played a significant role in the livelihood of the tribal communities, as they mainly prove to be of dietary and medicinal importance. Realising this significance, several ethnobotanical studies have been undertaken, to tap the specific nutraceutical and pharmaceutical potential **(Dhore et. al, 2012; Yesodharan and Sujana, 2007; Oak et. al, 2015; Deshmukh and Waghmode, 2011)**.

The study observations by a group of researchers viz. **Sonawane et al., 2012; Chotte et al, 2014; Oak et al 2015; Shivprasad et al 2016,** noted that there is a huge gap in understanding the importance and significance of this secondary information, and the

applications of the same. Some wild edible plants also have medicinal properties. Such dual roles of wild plants are common in the rural areas. Palghar district is well known for the WEP consumed by the tribal population.

Traditionally used wild edibles have enormous medicinal potential and could be an excellent source of nutrition and vitamins as a supplementary food. This immense knowledge gained through trial and error practices over several decades needs to be explored as they are an integral part of Indian culture.

In **2011, Padulosi et al** reported that out of an estimated portfolio of 30,000 edible plant species recorded worldwide, man depends only on 12 crops for the bulk of its nutritional requirements. This implies the great potential of wild edibles in the context of food security. The decreased use of wild edibles due to modernization and lack of knowledge among the younger generation are the major challenges. Thus it is important to conduct various studies on the commercial potential for ensuring the conservation and sustainable utilisation of these species to hold a food-secure future. **(Yatish Lele, et. al, 2003)** The disease preventive and health promoting approach based on herbal medicine takes into consideration, the promotion of health and treating ailments in a holistic way and finds increasing acceptability in many regions of the world, India and China being in the forefront. The practice of herbal medicine basically depends on folklore and on the experiences of people over a long period of time. However, very little research especially in the area of basic science has been conducted in a systematic manner. Medicinal plants with a long history of safe and effective use are likely to have a pharmaceutical effect. The use of plants in traditional medicine can be explained by physiologically active phytochemical compounds of a species. Plants produce a great diversity of substances that could be of therapeutic significance in many areas of medicine. However, the chemical nature of plant compounds present naturally as such and in extract, is puzzling. It is important to know constituents of plant extracts and their relative importance in their therapeutic value. Folk medicine is the mother of the healing systems of India **(Valiathan, 2006)**. It is universally accepted now that traditional health care systems have their unique strengths and are worth exploring for their potential preventive and curative properties. Traditional system of medi-care is still practised by the people of rural areas as they are

safe, have no adverse effects, inexpensive and ease availability. However, a gradual decline in practice of traditional herbal medicines has been reported in the recent past. Hence, there is an urgent need to investigate in detail the indigenous ethnobotanical knowledge held by each tribal people before such valuable knowledge disappears. Further, it is also necessary to conduct scientific investigations to understand the mechanism of action. **(S. Babu et. al, 2011)**

For a long time, medicinal plants have been used for the treatment of many infectious diseases without any scientific evidence. At present there is more emphasis on determining the scientific evidence and rationalisation of the use of these preparations. The use of herbal drugs for the prevention and treatment of various health ailments has been in practice from time immemorial. Many plants belonging to varied geography are being studied in large to identify the compounds of medicinal value. The crude extracts of many plants are reported to have a multi-beneficial effect on diseases and disorders. The relative efficiency of each compound and the possibilities of purifying them to homogeneity and developing a drug formulation is still beyond reach. Moreover, generally it is believed that the risk associated with herbal drugs is very less, but reports on serious reactions are indicating the need for development of effective marker systems for isolation and identification of the individual components. Standards for herbal drugs are being developed worldwide but as yet there is no common consensus as to how these should be adopted. Standardisation, stability and quality control for herbal drugs are feasible, but difficult to accomplish. The search for new molecules, nowadays, has taken a slightly different route where the science of ethnobotany and ethnopharmacology are being used as guides to lead the biotechnologist towards different sources and classes of compounds. With intense research on the herbal compounds from our huge natural wealth, the development of herbal medicine technology for every of our diseases and disorders is not far from now. **(S. Babu 2011)**

Nowadays, our world is in the anguish era of developing conventional therapeutics against cancer and multidrug resistant pathogens. Aside from naturally occurring medicinal plants and conventional therapeutics, physico-chemical properties of nanoparticles are proved to be a promising approach to enhance the efficacy of drugs against many epidemics. **(Kim, K.Y. 2007)**

The diversity of physico-chemical properties of different nanoparticles is the driving force behind nanotechnology and its application. The properties include high surface-to-volume ratio, surface potential, surface energy, crystallinity, catalytic effect, magnetic effect and so on. In spite of several physical and chemical methods for synthesising nanoparticles, increasing necessities of nanoparticles may be remunerated by the green chemistry method, as it does not use toxic chemicals or produce toxic byproducts in the process. Biofabrication or biosynthesis exploits biomolecules from plant extracts, bacteria, fungi, viruses and so on for the reduction of metal ions into an elemental product and to respective elemental metal nanoparticles **(Zhang M. et. al, 2016; Monica Dubey et. al, 2013).** In addition to medicinal properties, plants have phytochemicals, primary metabolites and secondary metabolites, which are primarily responsible for the reduction of metal ions into elemental metal nanoparticles. In addition, plants have a relatively higher nanoparticles yielding rate, easy availability, and lack the culture maintenance, as required in the microorganism dependent fabrications Plant extracts and their derivatives are widely known for their therapeutic potentials against many diseases. The activity of these compounds in vivo mainly depends upon the proper and controlled delivery to the target site. However, the therapeutic potential of plant extracts has been compromised due to the lack of controlled delivery of an effective dose to the target site. Hence, the plant extract mediated nanoparticles formulation may be a key solution to the limitation. **(Parth Sarthi Nayak et. al., December 2018)**

Nanoparticles fabricated using medicinal plant extract have great potential in the area of nanomedicine. High surface-to-volume ratio of nanoparticles enhances the local active biomolecules concentration, leading to many fold increases in the medicinal potentials. The silver nanoparticles (AgNPs) fabricated using indigenous medicinal plants of India, *Azadirachta indica* and *Syzygium cumini*, have shown a significant effect on the viability of prokaryotic and eukaryotic cells. (**Nayak PS, et. al., April 2019)**

Numerous studies tried to characterise phytochemicals with positive effects on the neural system from medicinal, and even dietary plants **(Kim et al., 2014; Naoi et al., 2017, 2019)**. Beneficial effects of neuroprotective phytochemicals are mainly attributed to their

antioxidant, and anti-inflammatory properties (Kim et al., 2010). The bioavailability of herbal bioactive components in body is a key point in their bioefficacy, and might be restricted by factors such as fast metabolism, trivial permeability, and the lack of stability within the CNS **(Pandareesh et al., 2015)**

Cur can modulate the inflammation process through suppression of several pro- and/or anti-inflammatory mediators such as tumour necrosis factor alpha (TNF-α), cyclooxygenase-2 (COX-2), and interleukin 8 (IL-8). Moreover, it has been reported that Cur is effective on Aβ and other protein aggregation, making it favourable to improve NDs, primarily AD **(Zhang et al., 2010; Hajialyani et al., 2018; Del Prado-Audelo et al., 2019)**. Despite its impressive therapeutic properties, Cur showed inappropriate pharmacokinetics, in terms of limited absorption, negligible bioavailability, and fast elimination from the body. To overcome such defects, various NPs, nanocapsules, nanomicelles and nanoliposomes were developed to improve the pharmacokinetics and the bioavailability of Cur **(Bollimpelli et al., 2016).**

When it comes to biocompatible nanoparticles among all green synthesis methods, plant extract mediated fabrication comes first for its cost effective and easy handling protocol. Interestingly, use of medicinal plants for fabrication instead of other plants has the possibility of garnishing the plant medicinal value onto the nanoparticle interface. Thus, the work used two different medicinal plants, known for their anti-pathogenic values in the Indian subcontinent, to fabricate AgNP, and to evaluate relative antipathogenic propensity of the particle compared to commercially available AgNP or plant extract individually. **(Ahmad, I., et. al., 2001)**

Antibiotics have been a staple of modern medicine's toolkit to fight illnesses, but antibiotic treatment has become less reliable as antimicrobial resistance (AMR) has evolved in many bacterial pathogens, causing the need for costly new treatments to be developed. The factors leading to such an increase are various. But the indirect role of antibiotics in this concern is a self-alarming issue. Most antibiotics are half a century old and the overuse of these drugs has created an acute antimicrobial resistance (AMR) crisis worldwide which is claiming a large number of lives, including in India. Antibiotic

resistant bacteria are bacteria that are not controlled or killed by antibiotics. They are able to survive and even multiply in the presence of an antibiotic. Most infection-causing bacteria can become resistant to at least some antibiotics. Bacteria that are resistant to many antibiotics are known as multi-resistant organisms (MRO). Some bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, *Staphylococcus aureus* ('golden staph' or MRSA) and *Neisseria gonorrhoeae* (the cause of gonorrhoea) are now almost always resistant to benzyl penicillin. In the past, these infections were usually controlled by penicillin.
India carries one of the largest burdens of drug-resistant pathogens worldwide. **(Gandra S, Klein EY et al., 2016)**

There is an urgent demand for an alternative antibacterial agent to reduce the drug resistance and environmental pollution caused by the abuse of antibiotics. The significance of nanotechnology lies in its vast industrial applications in biomedical and bioengineering fields. In recent decades, nanoparticles (NPs) have been recognized as one of the most cutting-edge materials. Many procedures are involved in the synthesis of NPs, such as heat evaporation, chemical reduction, biological, electrochemical, reverse micelle, thermal decomposition, radiation, and microwave-assisted strategies. Most of the techniques are time-consuming and require different hazardous chemicals to produce NPs. **(Farmoudeh A, Shokoohi A et. al., 2019)**

The World Health Organization (WHO) believes that 80% of the population of the world still uses traditional medicine, including plant-based medications as they have a variety of medical benefits. Currently, different antimicrobial agents are being developed for use on bacterial infections which are resistant to antibiotics. In addition to the development of new antibiotics, there is a need for alternate ways of treating various bacterial infections in order to overcome the high mortality rates because of high AMR among bacteria. **(Ramzan M, Karobari MI et. al., 2022)** Recent studies have shown that the use of colloidal systems to transport antimicrobial compounds enhances their therapeutic effects. **(Sadeghi Ghadi Z et al., 2019; Mir M,**

Ebrahimnejad P et. al., 2014)

Nanoparticles (NPs) protect the drug against environmental factors and bacterial enzymes. Also, by binding the appropriate compounds to the surface of the NPs, they will find the ability to target microorganisms that enhance the efficiency of treatment. Biocompatible and biodegradable polymers widely used in pharmaceutical formulations. In an acidic medium, the surface charge of polymers like Chitosan (CHI) is positive, and this property is used to formulate targeted NPs. Studies have shown that chitosan binding to lipopolysaccharides on the bacterial surface disrupts the order of the bacterial membrane and causes the plasma to leak out. It also increases the chances of drug penetration into the bacterial cytoplasm. **(Farmoudeh A, Shokoohi A et. al., 2021)** Plants also include bioactive secondary metabolites such as aldehydes, ketones, terpenoids, polyphenols, tannins, polysaccharides, flavonoids, proteins, amines, and alkaloids, which serve as stabilising and capping agents, and can reduce metal ions into metal nanoparticles, synthesising required NPs with previously reported beneficial properties **(Jalal, M et. al., 2018)**. As nanoparticles include plant extracts, animal proteins, agro-waste, pigments, bacteria, fungi, and small viruses, which are difficult to handle with conventional methods, green synthesis of nanoparticles is recommended **(Dos Santos, C.A et. al., 2014)**. Silver nanoparticles are among the most widely studied nanoparticles because they have flourished their wide applicability in the fields of biomedical applications, catalysis, electronics, and antimicrobial applications **(Jain, A et al., 2020).** The last decade has seen a rise in research in the field of green synthesis of silver nanoparticles owing to environmental concerns **(Jain, A et al., 2020)**.The actual situation of the pandemic fetched silver nanoparticles to the attention of researchers with special characteristics concerning antibacterial and antiviral protection **(Marinescu, L.; Ficai et. al., 2022)**. To broaden the knowledge in the field of green synthesis of nanoparticles that have catalytic activity, biosynthesis of silver nanoparticles using spent coffee grounds for catalytic degradation of an organic contaminant has also been reported. **(Mangindaan, D. et. al., 2020)** The properties of nanoparticles are exceptionally sensitive to their morphology, which increases the capability for their applications but at the same time makes their precise and

reproducible synthesis imperative **(Jain, A et al., 2020)**. Higher concentrations of silver are toxic; the literature shows that lower concentrations of $AgNO_3$ have better intrinsic therapeutic prospects, catalytic activity, chemical stability, and biocompatibility **(Akinola, P et. al., 2020).** Compared to bulk metals and their salts, AgNPs have been reported to have anticancer and antimicrobial activity **(Adebayo, E.A et. al., 2019)**. Among the best attributes of silver nanoparticles is the gradual and controlled release of silver. Moreover, **Padnya et al. 2020**, in their review, discussed the attractiveness of silver nanoparticles as an alternative to costly gold nanoparticles. AgNPs, apart from being used for medical purposes, are being exploited for their redox stability in biosensors with the assistance of cyclophanes.

In India, various actions have been taken including setting up of a National Task Force on AMR Containment (2010), "Chennai Declaration" by a consortium of the Indian Medical Societies (2012), Setting of Indian Council of Medical Research national surveillance network of laboratories, "Redline" campaign for educating public and National Action Plan on AMR 2017. The emergence of newer Antimicrobial resistant strains is posing a great challenge to India while India is still trying to eradicate issues related to tuberculosis, malaria and cholera pathogens. A lack of research and evidence compounds the problem.

With all of the available resources and information about the alarming situation existing around us, it becomes our moral obligation to contribute in any possible small or big though notable
way to address this problem of increasing Antibiotic Resistance of microorganisms. And when efforts are made to incorporate our own natural resources as a solution to this problem, it turns out to be an added advantage to reduce the time, energy and money for effective results.

A few of the ethnobotanical plants of the Palghar district from various research papers are enlisted below: (Table-1)

Sr. No.	Name.	Family	Local Name in the study area.	Edible and medicinal uses
1.	*Hygrophila auriculata* (Schumach.) Heine	Acanthaceae	Likhara, Vikhra, Talimkhana, Kolsunda, Ekhara	The crushed fresh leaves are applied on wounds, infection and headache. Leaves are traditionally used for the treatment for inflammation and edema, pain, urinary infection, diuretic, diarrhoea, dysentery, any abdominal diseases and constipation.
2.	*Celosia argentea* L.	Amaranthaceae	Kurdu, Kurada	Dry leaf powder mixed with honey is applied for skin infection. Fresh leaf paste is applied on wounds. The whole plant infusion is used for dysentery, diarrhea, acute abdominal pain, and inflamed stomach. The crushed leaves applied on sores, wounds, and boils and to reduce itching.
3.	*Hemidesmus indicus* (L.)	Apocynaceae	Ananthamul, Upalasari, Ananathavel Shutmali	Leaf extract is used for urinary infection.
4.	*Borassus flabellifer L.*	Arecaceae	Tadi, Tad, Tar	The juice from the flowering stalk mixed with boiled rice, keep for fermentation and this poultice is applied on wounds, ulcers and boils and other skin problems for healing.
5.	*Xanthium strumarium L.*	Asteraceae	Shankeshwara Duthundi, Lepadi, Ghagara	Young leaves are used as vegetables. Leaves, seeds, thorns or roots are used for toothache.
6.	*Impatiens balsamina L.*	Balsaminaceae	Cherda, Tivara, Terda,	The decoction of the dried plant is used for fungal and bacterial infection.

			Terada	
7.	*Basella alba L.*	Basellaceae	Mayalu, Poi , Velbendi	Leaf extract is used to heal boils, ulcers, headaches and skin allergy.
8.	*Commelina benghalensis L.*	Commelinaceae	Kena	The dry leaf powder mixed with warm water is given orally for diarrhoea. Leaf extract is applied for any skin infection and swelling.
9.	*Brassica rapa L.*	Brassicaceae	Sarso, Kali Mohari	A decoction of the leaves or stems or seeds is used in the treatment of cancer. Leaf and root extracts have antibacterial and antifungal properties.
10.	*Cheilocostus speciosus* (J. Koenig) C.D.Specht	Costaceae	Peva, Penva, Pushkarmula, Mohan, Pewda, Kosht, Harduli	The plant extract is used to treat cold, pneumonia, and rheumatism. Extract of young leaves and stems can be taken internally for diarrhoea, dysentery, for ear infections and eye infections. It can be used to treat cough and cold, congestion of the nose and lungs, and internal worms. Used externally to treat skin infections, scabies burns and ulcers.
11.	*Bryophyllum pinnatum (La m.) Oken*	Crassulaceae	Gayamari, Jambulchabi, Panphuti,	The leaf paste raw or slightly warmed is applied on boils, bruises, wounds, sores, and for insect bites.
12.	*Jatropha curcas L.*	Euphorbiaceae	Lepali, Chandanjot, Poki, Candrajot,	Leaves are used in the pesticide "das parni" used as spray. The twig is used as tooth brush for tooth ache. The white latex from leaves or stem is applied on teeth for tooth ache.
13.	*Abrus precatorius L.*	Fabaceae	Gunj, Gunja	Leaves are used in meeta pan, Leaves and flowers are edible Grind the leaves and apply for skin diseases.

14.	*Bauhinia variegata L.*	Fabaceae	Kachanar, Koharel, Kanchan, Kovidara	The decoction of the leaves is given to reduce headaches. Decoction of dried buds and leaves is given orally for piles and dysentery and diarrhoea.
15.	*Lablab purpureus (L.)* Sweet	Fabaceae	Sem, Val- papadi, Pandhrepavate Popat, Kadavebala.	The leaves are used in the treatment of cholera, vomiting, diarrhoea, leucorrhoea, gonorrhoea,.
16.	*Tridax procumbens L.*	Asteraceae	Kamber modi	Whole plant made into paste and taken orally in diarrhoea.

RATIONALE

The proposed study will be focussed on studying the antibacterial effect of Plant derived Nanoparticles (PDNPs) on some pathogenic bacterial cultures. The study intends to synthesise nanoparticles using the plant extracts of various documented traditional and ethnobotanical significant plants of Palghar district.

India is a country rich in its biodiversity throughout all different states and regions. With the gradual loss of traditional/tribal plants and the constant decline in the traditional knowledge of various medicinal plants with the passing generations, the ethnobotanical studies in India have recently gained momentum. These studies have led to the documentation of various indigenous varieties of plants in the Palghar district also and covers up nearly 18 talukas in the North-Western part of Maharashtra, India.

Analytical and antibacterial research studies of such significant plants can open possibilities in combating various medical conditions with cost-effective and beneficial solutions. From the various literatures published we are aware that India carries one of the largest burdens of drug-resistant pathogens worldwide, including the highest burden of multidrug-resistant tuberculosis, alarmingly high resistance among Gram negative and Gram-positive bacteria even to newer antimicrobials such as carbapenems and faropenem since its introduction in 2010. According to the reports published in the magazine *'Down to Earth'* two million deaths are projected to occur in India due to AMR by the year 2050.

Based on the aforementioned documentation of the ethnobotanical research work, we can understand that there are a variety of wild edible plants that have medicinal uses. Such plants can be explored for their phytochemical analysis and antibacterial activity. Some of the plants as tabulated in the Table-1 have medicinal applications to be explored further.

Lack of focused study has led to the occurrence of increased mortality due to deadly pathogens creeping into our day-to-day lives. The effect of various traditional plants on the disease healing in the Palghar area has not been studied enough. AMR being a silent pandemic is an alarming issue to be tackled and the problem lies in the lack of research data. The need to take up research activities and study the polluted resources

is a next major step to analyse and evaluate the effect of traditional knowledge in order to develop better medical treatments.

Also research on the antibacterial effect of various ethnobotanical plant extracts of plants in the Palghar region need a thrust to utilise the rich biodiversity of this region. The intent of this research is also to transform the traditional knowledge to technological application. The benefits of developing nanoparticles or nanomolecules lies in its structural and functional characteristics. Nanoparticle synthesis has been achieved using chemical and physical techniques; however, these methods are not beneficial to the environment. The COVID-19 pandemic also drew the attention of various researchers on the antibacterial and antiviral applications of silver nanoparticles. Silver nanoparticles are the most widely used type of nanoparticles due to its various properties. But if its use is uncontrolled and is chemically synthesised, it raises an environmental concern. Also, as nanoparticles are difficult to handle with conventional methods, green synthesis of nanoparticles is recommended. Also plant extracts can easily reduce the metal ions and be used as capping agents to synthesise effective, eco-friendly nanoparticles. The nano size of these molecules makes it quicker to penetrate and attack the target cells. The scientific fraternity and the government authorities need to take co-operative measures in order to address the present situation of antimicrobial resistance in humans, animals as well as the environment. All these components of the ecosystem being interrelated, it poses an even greater challenge if reliable research data remains unavailable.

The present research aims to study the effect of various plant derived nanoparticles from the local plants on some pathogenic bacterial cultures. The organisms that are gaining resistance to the current drug treatments are on an ever increasing pace. The study may widen horizons to develop possible solutions in the medical and environmental issues.

The present research will also help to understand and apply the traditional knowledge treasured by the local and tribal people of the Palghar region. Also, this study will help to see the prospects of green synthesis of nanoparticles from ethnomedicinal plants as a

potential therapy. The research will enable us to study and gain a clear insight on the effect of pollution due to waste disposal into the water bodies posing a threat to the health condition of the residents and general public of a particular coastal region.

By connecting **'tradition to technology'** the research intends to present a cost-effective and reliable solution to the ever-increasing medical and environmental threat of the developing country. The merging of traditional knowledge and effective technology will prove to be effective in various bacterial infections and open up an effective method of applying the science in day-to-day lives.

AIMS & OBJECTIVES OF THE STUDY

AIMS:

1. To collect the ethnobotanical plant samples from the tribal region of Palghar district.
2. To authenticate the collected ethnobotanical plants of the Palghar region and synthesise nanoparticles from the plant part extracts.
3. To characterise the synthesised nanoparticles.
4. To study the antibacterial effect of the synthesised nanoparticles on the pathogenic bacterial cultures.

OBJECTIVES:

1. **Collection of plant samples and derive plant extracts to perform the phytochemical analysis.**
    (a) To collect the ethnobotanical significant plant samples and authenticate the plant species.
    (b) To obtain extracts from the different plant samples known for their traditional uses by the tribal communities of Palghar district.
    (c) Qualitative and Quantitative analysis of phytochemical constituents in the plant extracts.
2. **Green Synthesis of nanoparticles and their characterisation.**
    (a) To synthesise the AgNPs by using plant extracts obtained from the various plant samples.
    (b) To characterise the synthesised nanoparticles using the various techniques like UV-Vis Spectrophotometry/HPLC/XRD/SEM
3. **Determine the antibacterial activity of the newly synthesised PDNPs on the bacterial isolates in comparison with standard antibiotics.**
    (a) To study the inhibitory action of the plant derived nanoparticles on the bacterial isolates at varying concentration.
    (b) To draw a comparison of efficacy of the PDNPs and the existing common antibiotics.

PLAN OF WORK OF STUDY:

Research studies on the varied ethnobotanical significant plants used among the tribal communities of the different talukas of the Palghar district have been carried out. The study documentation of the indigenous plants by various researchers have been limited to the listing and medicinal uses only.

The scientific research on the antibacterial activity of these plants will open up new vistas to tap the potential of the native plants and the traditional knowledge in modern day therapeutics.

The proposed study aims to bridge this gap by carrying out the in vitro studies and lay a foundation for further research opportunities and technological applications of the traditional treasure of our country.

A) Sampling and analysis of traditional plant varieties.

1. The ethnomedicinal significant plant varieties will be collected from the tribal areas of the Palghar district.
2. Determining the primary data of physiological parameters: The physical characteristics of the obtained plant varieties will be studied and analysed for further study.
3. Desired plant parts (leaf/root/stem) will be sampled. This selection of the desired plant part will be based on the documentation in the various research papers.

B) Laboratory Preparation of Extracts from Plants.

1. The desired plant parts will be cleaned, sun-dried and ground for obtaining the powdered form of the sample.
2. The powdered samples will be processed further to obtain the filtered aqueous/methanolic/ethanolic extracts. The extraction method and use of solvent to get the maximum extract will be determined by this step.
3. This will be further refrigerated and used as required to synthesise AgNPs.

C) Synthesis and characterisation of PDNPs (Plant derived nanoparticles)

1. The extracts obtained from the plants will be treated with $AgNO_3$ solution and incubated as required for the synthesis of PDNPs.
2. The synthesised PDNPs will be analysed and characterised for their properties by the UV-Vis/FTIR/XRD/SEM methodology.

D) Determine the efficacy of the PDNPs on the pathogenic bacterial strains.

1. The newly synthesised PDNPs will be diluted to different concentrations to obtain the efficacy of the nanoparticles on the standard pathogenic strains.
2. Comparative analysis of the newly synthesised NPs with the antibiotics widely used against the bacterial isolates.
3. Appropriate controls for the standards will be maintained.
4. Recording and Analysis of the observations.

INSTRUMENTS and APPARATUS TO BE USED IN THE STUDY

1. ***UV Visible Spectroscopy*:**

   The most important and simple technique for confirming the formation of nanoparticles is ultraviolet visible (UV Vis) spectrophotometry. AgNP formation will be verified using a UV visible spectrophotometer.

2. ***X-ray Diffraction (XRD) Analysis:***

   The crystalline nature of the biosynthesized AgNPs will be analysed using X-ray diffraction (XRD).

3. ***Fourier Transform InfraRed (FTIR) Spectrum:***

   The Fourier Transform InfraRed Spectrophotometer will be used to determine the functional groups responsible for the synthesis of silver nanoparticles and record the FTIR spectrum. These functional groups may aid in the capping, reduction, and stabilisation of silver nanoparticles.

4. ***Scanning Electron Microscope Analysis (SEM):***

   To determine the structure of the obtained nanoparticles, SEM will be used.

EXPECTED OUTCOME

Nanoparticles have a more germicidal effect than the mass of silver metal because of the reduction of size, increase of the ratio of surface to volume of nanoparticles, and increase of the contact area with microorganisms. This study may propagate the simple use of a natural, low-cost biological reducing agent and plant extracts through efficient green nanochemistry methodology, avoiding the presence of toxic solvents and waste. The study on antibacterial activity of the PDNPs from the tribal plant extracts may be a boon to develop novel therapeutic remedies without deleterious effects on the consumer as well as the environment.

CONTRIBUTION TO THE SOCIETY

- Silver nanoparticles made from traditional plant extract are expected to be more effective against human infections as compared to the plain plant extracts. The antibacterial activity study will be an additive source to propagate the application and thereby the conservation of traditional biodiversity. Because of these applications, this technology is potentially promising for the large-scale production of nanoparticles. Prepared nanoparticles can be employed as antibacterial agents in wound healing, water purification, and medicine.
- The use of these indigenous plants can be effectively made in the modern day therapy against various diseases caused by varied bacterial strains. Also, the tribal communities may be encouraged and provided amenities to conserve the biodiversity of the tribal regions.
- The proposed research will create a social awareness about the preventive measures and actions to be taken in order to avoid the spread of threatening diseases. It will enable us to understand the necessity of research on this issue throughout the small and big towns in India. The application of simple, known, harmless alternatives to alleviate the indiscriminate prescription of modern medicines can be an encouraging factor.

FUTURE PROSPECTS

- The effectiveness of traditional medicines with improved technological administration will help in easy and cost-effective control measures.
- The project will also help to improve the quality of life of the native population of Palghar district. This research will also help to expand horizons of scientific research and contribute to the National Action Plans.
- Commercialisation of the plant products will improve the quality of life (QoF) of the tribal communities.

REFERENCES

1. Taneja N, Sharma M. Antimicrobial resistance in the environment: The Indian scenario. Indian J Med Res. 2019 Feb;149(2):119-128. doi: 10.4103/ijmr.IJMR_331_18. PMID: 31219076; PMCID: PMC6563737.

2. Dixit A, Kumar N, Kumar S, Trigun V. Antimicrobial Resistance: Progress in the Decade since Emergence of New Delhi Metallo-β-Lactamase in India. Indian J Community Med. 2019 Jan-Mar;44(1):4-8. doi: 10.4103/ijcm.IJCM_217_18. PMID: 30983704; PMCID: PMC6437806.

3. Das B, Chaudhuri S, Srivastava R, Nair GB, Ramamurthy T. Fostering research into antimicrobial resistance in India. BMJ. 2017;358:j3535. [PMC free article] [PubMed] [Google Scholar]

4. Global burden of bacterial antimicrobial resistance in 2019:a systemic analysis,vol.399, issue 10325, pg. 629-655, Feb.12, 2022 https://doi.org/10.1016/S0140-6736(21)02724-0

5. Zhang M, Viennois E, Prasad M, Zhang Y, Wang L, Zhang Z, Han MK, Xiao B, Xu C, Srinivasan S, Merlin D. Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials. 2016 Sep; 101:321-40. doi: 10.1016/j.biomaterials.2016.06.018. Epub 2016 Jun 9. PMID: 27318094; PMCID: PMC4921206.

6. Walia K, Madhumathi J, Veeraraghavan B, Chakrabarti A, Kapil A, Ray P, Singh H, Sistla S, Ohri VC. Establishing Antimicrobial Resistance Surveillance & Research Network in India: Journey so far. Indian J Med Res. 2019 Feb;149(2):164-179. doi: 10.4103/ijmr.IJMR_226_18. PMID: 31219080; PMCID: PMC6563732.

7. Ramzan M, Karobari MI, Heboyan A, Mohamed RN, Mustafa M, Basheer SN, Desai V, Batool S, Ahmed N, Zeshan B. Synthesis of Silver Nanoparticles from Extracts of Wild Ginger (Zingiber zerumbet) with Antibacterial Activity against Selective Multidrug Resistant Oral Bacteria. Molecules. 2022 Mar 21;27(6):2007. doi: 10.3390/molecules27062007. PMID: 35335369; PMCID: PMC8949094.

8. Sadeghi Ghadi Z, Ebrahimnejad P. Curcumin entrapped hyaluronan containing niosomes: preparation, characterisation and in vitro/in vivo evaluation. J Microencapsul. 2019;36(2):169–79. doi: 10.1080/02652048.2019.1617360. [PubMed] [CrossRef] [Google Scholar]

9. Sadeghi Ghadi Z, Dinarvand R, Asemi N, Talebpour Amiri F, Ebrahimnejad P. Preparation, characterization and in vivo evaluation of novel hyaluronan containing niosomes tailored by Box-Behnken design to co-encapsulate curcumin and quercetin. Eur J Pharm Sci. 2019;130:234–46. doi: 10.1016/j.ejps.2019.01.035. [PubMed] [CrossRef] [Google Scholar]

10. Mir M, Ebrahimnejad P. Preparation and characterization of bifunctional nanoparticles of vitamin E TPGS-emulsified PLGA-PEG-FOL containing deferasirox. Nanoscience & Nanotechnology-Asia. 2014;4(2):80–7 doi: 10.2174/2210681205666150515000224.

11. Farmoudeh A, Shokoohi A, Ebrahimnejad P. Preparation and Evaluation of the Antibacterial Effect of Chitosan Nanoparticles Containing Ginger Extract Tailored by Central Composite Design. Adv Pharm Bull. 2021 Sep;11(4):643-650. doi: 10.34172/apb.2021.073. Epub 2020 Sep 8. PMID: 34888211; PMCID: PMC8642796.

12. Gandra S, Klein EY, Pant S, Malhotra-Kumar S, Laxminarayan R. Faropenem consumption is increasing in India. Clin Infect Dis. 2016;62:1050–2. [PubMed] [Google Scholar]

13. Shivangi Chaudhari,Dakshata Patil, Jayananda Tosh, Traditional Ethnomedicinal Plants Used By the Tribal in Coastal Area of Dahanu Taluka, Palghar District, Maharashtra State, India, VOLUME-6 | ISSUE-2 | FEBRUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

14. Dr. Jayananda Tosh, NUTRITIVE EVALUATION OF 18 PROMISING WILD EDIBLE AND FODDER LEGUMINOUS SPECIES OF WESTERN MAHARASHTRA, Paripex - Indian Journal Of Research, Volume-7 | Issue-3 | March-2018 | ISSN - 2250-1991 | IF : 6.761 | IC Value : 86.18

15. Jalal, M.; Ansari, M.A.; Alzohairy, M.A.; Ali, S.G.; Khan, H.M.; Almatroudi, A.; Raees, K. Biosynthesis of silver nanoparticles from oropharyngeal Candida glabrata isolates and their antimicrobial activity against clinical strains of bacteria and fungi. Nanomaterials 2018, 8, 586.

16. Dos Santos, C.A.; Seckler, M.M.; Ingle, A.P.; Gupta, I.; Galdiero, S.; Galdiero, M.; Gade, A.; Rai, M. Silver nanoparticles: Therapeutical uses, toxicity, and safety issues. J. Pharm. Sci. 2014, 103, 1931–1944. [CrossRef]

17. Marinescu, L.; Ficai, D.; Ficai, A.; Oprea, O.; Nicoara, A.I.; Vasile, B.S.; Boanta, L.; Marin, A.; Andronescu, E.; Holban, A.-M. Comparative Antimicrobial Activity of

Silver Nanoparticles Obtained by Wet Chemical Reduction and Solvothermal Methods. Int. J. Mol. Sci. 2022, 23, 5982.

18. Jain, A.; Malik, A.; Malik, H.K. Mathematical modelling of seed-mediated size-specific growth of spherical silver nanoparticles using Azadirachta indica leaf extract. J. Taibah Univ. Sci. 2020, 14, 873–880. [CrossRef]

19. Mangindaan, D.; Lin, G.-Y.; Kuo, C.-J.; Chien, H.-W. Biosynthesis of silver nanoparticles as catalyst by spent coffee ground/recycled poly (ethylene terephthalate) composites. Food Bioprod. Process. 2020, 121, 193–201.

20. Akinola, P.; Lateef, A.; Asafa, T.; Beukes, L.; Hakeem, A.; Irshad, H. Multifunctional titanium dioxide nanoparticles biofabricated via phytosynthetic route using extracts of Cola nitida: Antimicrobial, dye degradation, antioxidant and anticoagulant activities. Heliyon 2020, 6, e04610. [CrossRef]

21. Adebayo, E.A.; Ibikunle, J.B.; Oke, A.M.; Lateef, A.; Azeez, M.A.; Oluwatoyin, A.O.; Ayanfe Oluwa, A.V.; Blessing, O.T.; Comfort, O.O.; Adekunle, O.O. Antimicrobial and antioxidant activity of silver, gold and silver-gold alloy nanoparticles phytosynthesised using extract of Opuntia ficus-indica. Rev. Adv. Mater. Sci. 2019, 58, 313–326. [CrossRef]

22. Padnya, P.; Gorbachuk, V.; Stoikov, I. The Role of Calix[n]arenes and Pillar[n]arenes in the Design of Silver Nanoparticles: Self-Assembly and Application. Int. J. Mol. Sci. 2020, 21, 1425

23. Nayak PS, Pradhan S, Arakha M, Kumar D, Saleem M, Mallick B, Jha S. Silver nanoparticles fabricated using medicinal plant extracts show enhanced antimicrobial and selective cytotoxic propensities. IET Nanobiotechnol. 2019 Apr;13(2):193-201. doi: 10.1049/iet-nbt.2018.5025. PMID: 31051451; PMCID: PMC8676497.

24. Singh SP, Tripathi S, Shukla RS. Ethnomedicinal heritage for bioprospecting and drug development in North Eastern states of India. J Eco & Taxo Bot 2003; 27(2):384-395.

25. Ashidi JS, Houghton PJ, Hylands PJ, Efferth T. Ethnobotanical survey and cytotoxicity testing of plants of South-western Nigeria used to treat cancer, with isolation of cytotoxic constituents from Cajanus cajan Millsp. leaves. J Ethnopharmacol 2010; 128(2):501-512

26. Roy, Arpita. (2020). Plant derived silver nanoparticles and its therapeutic applications. Current Pharmaceutical Biotechnology. 21. 10.2174/1389201021666201027155708.

27. S. Babu, L. M. and. (2011). Phytotherapy in India: transition of tradition to technology. Current Botany, 2

28. Yatish Lele, Bhargavi Thorve, Swati Tomar, Anjali Parasnis, Wild Edible Plants of Nutritional and Medicinal Significance to The Tribes of Palghar, Maharashtra, India, Journal of Ecological Society, 10.54081/JES.027/03

29. Kim, K.Y.: 'Nanotechnology platforms and physiological challenges for cancer therapeutics', Nanomed. Nanotechnol. Biol. Med., 2007, 3, (2), pp. 103–110

30. Parth Sarthi Nayak , Stuti Pradhan , Manoranjan Arakha , Dileep Kumar , Mohammed Saleem , Bibekanand Mallick , Suman Jha; Silver nanoparticles fabricated using medicinal plant extracts show enhanced antimicrobial and selective cytotoxic propensities, December 2018, ISSN 1751-8741, doi: 10.1049/iet-nbt.2018.5025

31. Pradhan S, Arakha M, Kumar D, Saleem M, Mallick B, Jha S. Silver nanoparticles fabricated using medicinal plant extracts show enhanced antimicrobial and selective cytotoxic propensities. IET Nanobiotechnol. 2019 Apr;13(2):193-201. doi: 10.1049/iet-nbt.2018.5025. PMID: 31051451; PMCID: PMC8676497.

32. Ahmad, I., Beg, A.Z.: 'Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens', J. Ethnopharmacol., 2001, 74, (2), pp. 113–123

33. https://pib.gov.in/PressReleasePage.aspx?PRID=1848805

34. https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/

35. https://www.deccanherald.com/science-and-environment/the-crisis-of-antimicrobial resistance-in-india-1082420.html

36. https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/antibiotic-resistan t bacteria

37. https://health.economictimes.indiatimes.com/news/industry/amr-in-india-can-gain-of function-gof-research-prevent-the-next-pandemic/89547107

38. https://www.cseindia.org/page/antimicrobial-reststance-programme

39. https://www.downtoearth.org.in/news/antimicrobial-resistance-to-cost-global-econom y-up to-us-210-trillion-47948

40. Gazetteer of India - Maharashtra State - Thane district https://cultural.maharashtra.gov.in/english/gazetteer/Thane/Scheduled%20Tribes.html

41. Ghosh S, Varerkar SA. Undernutrition among tribal children in Palghar district, Maharashtra, India. PLoS One. 2019 Feb 27;14(2):e0212560. doi: 10.1371/journal.pone.0212560. PMID: 30811462; PMCID: PMC6392283.

42. Hala M. Abdelmigid, et. al.,2021, Comparative Analysis of Nanosilver Particles Synthesized by Different Approaches and Their Antimicrobial Efficacy, Hindawi Journal of Nanomaterials Volume 2021, Article ID 2204776, 12 pages https://doi.org/10.1155/2021/2204776